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Immune response to oral polio vaccine in patients with IgA glomerulonephritis

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SUMMARY

Oral poliovirus vaccine was used to immunize 51 patients with IgA glomerulonephritis and 44 healthy controls. The patients showed an enhanced antibody response compared to controls. This was apparent in a higher frequency of strong increases in neutralizing antibody titres during the course of immunization as well as higher levels of virus-specific IgA-class antibodies. IgG-class antibodies showed similar activity in both groups. In patients the neutralizing antibodies correlated with the virus-specific IgA-class antibodies, suggesting that the IgA-antibodies synthesized by the patients are functionally competent antibodies

Keywords IgA glomerulonephritis oral immunization polio vaccination

INTRODUCTION

Deposition of IgA-antibody-antigen complexes within the renal mesangium has been suggested as the mechanism of the kidney lesions observed in IgA glomerulonephritis (IgA-GN). Since the IgA molecules that are deposited in the kidney tissue are for the most part oligomeric it has been suggested that the complexes are formed within the mucosal membranes and transported to the kidneys (Bene, Faure & Duheille, 1982; Tomino *et al.*, 1982). A recent animal model supports this (Lamm, Emancipator & Gallo, 1984).

However, in an earlier report (Pasternack, Mustonen & Leinikki, 1986) we showed that IgA-GN patients respond to a parenteral vaccination with an augmented IgA-class antibody response. In this case inactivated mumps virus was used as the antigen. Similar results have been reported following the administration of influenza HA vaccine (Endoh *et al.*, 1984). These studies suggest that IgA-GN patients show an antigen-specific alteration of IgA response. We wanted to study whether this alteration depends on the parenteral administration of the foreign antigens or if it can be provoked by conditions simulating natural infections. Here we report that even when the immunological reaction follows peroral inoculation of attenuated virus, augmentation of antigen specific IgA-response occurs, suggesting that it can be provoked by very different antigenic structures and via very different routes of inoculation.

MATERIALS AND METHODS

In connection with an outbreak of paralytic poliomyelitis an extensive vaccination campaign was launched in Finland during spring 1985. Patients with IgA-GN and their healthy controls were

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vaccinated with oral poliovirus vaccine and their immune response was followed from serially collected blood samples.

Fifty-one patients with biopsy-proven IgA-GN and 44 healthy controls were enrolled in this study. The mean age and sex distribution in both groups was similar. None of the patients with IgA-GN had Henoch-Schönlein syndrome or chronic liver disease. The known duration of the nephropathy varied from less than 1 to 30 years. None of our patients had impaired renal function or the nephrotic syndrome. Patients and controls were given one single dose of standard oral poliovirus vaccine (Sabin). Blood samples were drawn at vaccination and two and four weeks later.

Antibody assays

Neutralizing antibodies. The standard method of microneutralization was used. One hundred $TCID_{50}$ of poliovirus type 3 virus (Strain Saucett, National Poliovirus Reference Laboratory, National Institute of Public Health, Helsinki, Finland) was incubated with serial dilutions of patient serum (1/10, 1/40, 1/60, 1/640 and 1/2560). After 1 h the serum-virus mixture was applied in two parallel microtitre wells where Vero-cells had been grown into monolayers. After 1 h maintenance medium (MEM) 2% newborn calf serum was added and the plates were incubated at 37°C. The developing CPE (cytopathic effect) was evaluated 48 h later. Neutralizing antibody levels are expressed as titres from the last dilution inhibiting the virus-induced CPE.

Immunoglobulin-class-specific antibodies. A standard 4-layer micromethod of ELISA was used (Turunen, Vuorio & Leinikki, 1983). The antigen was produced by growing the virus in Vero cells in roller bottles. After complete CPE, cells were detached, supernatant clarified by low speed centrifugation and virus collected by an MSE-2 ultracentrifuge at 100,000 *g* for 1 h through a 30% sucrose solution (5 ml sucrose for 28 ml of supernatant). The virus pellet was incubated with MEM + 0·5% SDS (sodium docedyl sulphate, Sigma) (1 ml/pellet) overnight at room temperature, followed by sonication (MSE, full stroke 1 min).

The virus was then stored in aliquots at −20°C. Microtitre plates were sensitized by the virus diluted 1:500 in 0·1 M carbonate buffer, pH 9·5. Sera and conjugates were diluted with phosphate-buffered saline supplemented with 0·1% Tween-20 and 1% bovine serum albumin. Class-specific anti-human immunoglobulin from rabbits was used as the third layer and HRPO-conjugated anti-rabbit immunoglobulin (all purchased from Dako, Denmark) as the fourth.

In each series known positive and negative standards were included and the results expressed as relative units, an average positive serum giving a value of approximately 100 units and a negative serum O (Turunen *et al.*, 1983).

Serum immunoglobulins and complement. Serum IgG, IgA, IgM, C3 and C4 were studied by standard methods using laser nephelometry (Sieber & Gross, 1977).

Statistical methods

For statistical analysis BMDP statistical software was employed. Antibody levels in the different groups were compared by two-tailed Mann-Whitney *U*-test and in other analyses χ^2 statistics were used.

RESULTS

Neutralizing antibodies. Every individual had received inactivated poliovirus vaccine according to the standard vaccination programme in Finland before the present study. However, more than 50% had no detectable antibodies at 1/10 dilution of their first serum sample. Twenty-six of the patients and 20 of the controls showed a significant (at least a 4-fold) increase in neutralizing antibody titres during the observation period (Table 1). The strength of the response as judged from the observed change in titre did not seem to correlate with the initial antibody levels: significant changes were also seen in cases whose initial antibody levels were as high as 160. IgA-GN patients had significantly more often strong (16-fold or greater) antibody response than the controls (Table 1).

Immunoglobulin-class specific antibodies. The initial levels of IgG-class antibodies were quite

Table 1. Change in serum neutralizing antibody titres following oral poliovirus vaccination in patients with IgA glomerulonephritis and in controls

Difference in titres	Number of subjects		Statistical significance*
	IgA-GN	Controls	
4-fold increase	12	14	NS
8-fold increase	3	3	NS
16-fold increase	11	3	$P<0.001$

* χ^2 test.
NS, not significant.

Table 2. Serum antibody levels in IgA-GN patients and in controls before vaccination (I), 2 weeks (II) and 4 weeks (III) after vaccination.

Sample	Test	Mean values (EIU)		Statistical significance*
		IgA-GN	Controls	
I	ELISA IgG	90·5	90·8	NS
	ELISA IgA	62·3	31·6	$P<0.001$
	NT	220	150	NS
II	ELISA IgG	99·4	99·3	NS
	ELISA IgA	67·5	32·6	$P<0.001$
	NT	454	276	NS
III	ELISA IgG	101·6	103·7	NS
	ELISA IgA	68·6	39·5	$P<0.001$
	NT	651	552	NS

ELISA, enzyme-linked immunosorbent assay; NT, neutralization test.
* Tested by Mann-Whitney test.

similar in patients and controls, suggesting that the patients and the controls had had more or less the same previous exposure to poliovirus antigens before this study. Also in later samples the mean IgG levels were similar in both groups. However, IgA antibody levels were already initially significantly higher in patients and remained so also in the later samples (Table 2). The IgA response was also stronger in the patients: 13 of them showed an increase of more than 20 units as against only five among the controls showing a similar change. The change in IgA antibody levels was relatively more often seen among patients aged more than 45 years. The initial antibody levels showed no correlation to the strength of the response.

Although in a multivariant analysis neutralizing antibody titres and IgG- and IgA-class antibody levels as measured by ELISA correlated significantly, in individual cases a significant increase in neutralizing antibody titres was not always followed by a significant change in ELISA-antibody levels or vice versa. The total number of persons having a significant increase in none of the three antibody categories was 18 among the patients and 17 among the controls. Interestingly, the patients had a significant IgA response or a combination of significant IgA-IgG plus

Table 3. Frequency of significant increases in antibody levels as measured by IgA- and IgG-class-specific ELISA and serum neutralization

Test	Number of subjects showing significant increases		Statistical significance*
	Patients	Controls	
ELISA IgA only	6	1	$P<0{\cdot}001$
ELISA IgG only	5	6	NS
Neutralization only	9	6	NS
All three tests	5	1	$P<0{\cdot}01$
Any test	33	24	NS

* χ^2 test.

neutralizing antibody response significantly more often than the controls (Table 3). In a statistical correlation matrix another interesting observation was made; in the patients but not in the controls a significant correlation was evident between the highest neutralizing titre and specific IgA antibody level not only in the third serum sample but also in the first, prevaccination sample (Cronbach's alpha test).

IgM-class antibodies were not observed either in the controls or in IgA-GN patients.

Serum immunoglobulins and complement. There were no differences in the levels of serum IgG, IgM, C3 and C4 between the patients and the controls. Total serum IgA levels were higher in the patients than in the controls in all three samples ($P<0{\cdot}01$). No significant changes were seen between the successive serum samples in any of these parameters in either group.

DISCUSSION

IgA glomerulonephritis is thought to result from an overproduction of IgA-antibody-antigen complexes on mucous membranes, which are then deposited in glomeruli. Serum sickness type pathogenesis following gastrointestinal exposure to antigens has been suggested (Lamm *et al.*, 1984) based on assumptions from animal models. However, earlier studies on humans have suggested that parenterally administered antigens, including influenza virus hemagglutinin and inactivated mumps virus (Endoh *et al.*, 1984; Pasternack *et al.*, 1986) result in augmented IgA-antibody response in these patients. Furthermore, clinical experience suggests that infections, including viral, are possible provoking factors of the disease. Here we have shown that perorally active antigens also result in a similar response suggesting that the route of immunization per se does not affect the dominant IgA-response in these patients. Since antigen presention and other circumstances leading to the activation of immune reaction must be quite different following orally administered live poliovirus than, parenteral administration of glycoprotein rich virus, our observation suggests that the step leading to altered IgA-response is beyond the primary activation of the immunocompenent T cell. Further studies *in vitro* using defined antigens are needed to elucidate the exact mechanism and the site of alteration.

The role of peroral immunogens has previously been evaluated by Sancho *et al.* (1983), who studied the response of IgA-GN patients to food challenge. Oral poliovirus vaccine may enter the circulation in nonimmune persons, but in immune individuals the virus can only replicate in the mucosal tissues of the small intestine. All our patients and controls had earlier received inactivated vaccine and can be regarded as immune in this respect. In spite of this, several individuals in both groups showed a significant increase in antibody levels after vaccination, demonstrating the good applicability of oral poliovirus vaccine to immunization studies like this. Also the observation that

the significant increases in neutralizing antibodies did not depend on the pre-existing antibody levels suggests that the antibody response is due to a local antibody stimulus in the gut where the virus replicates.

Patients with IgA-GN responded with an augmented IgA antibody increase after oral poliovirus vaccination. It is plausible that the enhanced neutralizing antibody titres are also due mostly to the IgA response. The observed correlation between neutralizing antibody response and levels of specific IgA antibodies in the patients further supports this.

In our earlier study with parenterally administered mumps virus vaccine, IgA-GN patients also showed a higher and more sustained IgG antibody response compared to healthy controls, whereas IgM responses were identical (Pasternack *et al.*, 1986). In the present work no such augmented IgG response could be observed. This is interesting and may be related to the different routes of antigen administration used in our vaccination studies. Similar findings have previously been made by another group (Emancipator, Gallo & Lamm, 1983). In their model, employing experimental animals, they found that oral immunization led to IgA antibody formation in the absence of IgG and IgM antibodies. The difference may also be antigen-specific: in the study of Endoh *et al.* (1984) no augmented IgG response could be documented in IgA-GN patients to parenterally given influenza vaccine.

In our previous study we also observed a decreasing complement level (C3 and C4) after parenteral mumps virus vaccine (Pasternack *et al.*, 1986). In the present work this could not be repeated. Again, a difference in the antigen and the route of administration may result in differences in the type of immune response provoked, as have been observed in experimental works (Emancipator *et al.*, 1983; Emancipator & Lamm 1984).

A significant IgA-class antibody response was found more often in patients over 45 years of age than in other groups. The reason for this is unknown. Also the total levels of serum IgA rise with increasing age in patients with IgA-GN (Mustonen *et al.*, 1985).

The mechanism by which IgA-GN patients overproduce IgA antibodies is not clear. IgA-specific T-helper cells have been found to be increased and IgA-specific suppressor T-cell activity decreased (Sakai *et al.*, 1982; Egido *et al.*, 1983). Our studies suggest that IgA-GN patients do display an overreaction in their IgA antibody response, which can be demonstrated with a variety of antigens and routes of administration. However, the triggering antigen may be individually variable, since not all of our patients were high responders. The same was observed by Endoh *et al.* (1984). The present study further suggests that the oral route of immunization, although important in provoking IgA response in general, is not different from the parenteral route in selecting out IgA-GN patients. With both routes of immunization the same frequency is seen of individuals showing enhanced IgA response to the antigen. Also the degree of immunological response seems to be independent of the route of antigen administration.

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